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(54)【発明の名称】　核酸増幅用の安定化された酵素組成物

(57)【要約】
核酸増幅において用いるための安定化された酵素組成物。単一の安定化された配合物中の１種類またはそれ以上の酵素の安定化のための組成物を提供する。更に別の組成物は、乾燥した安定化された酵素混合物と、必要な副因子および酵素基質とを一緒に、再水和の際に用いるための単一容器中に包含する。更に、安定化された酵素組成物の製造方法および使用方法並びに開示された組成物を包含する核酸増幅用のキットを開示する。

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
1. Approach of Storing and Stabilizing Enzyme Constituent in Single Container -- it is -- (a) and (I) Reverse Transcriptase and Active Oxygen Constituent Chosen from Group Which Changes from Reverse Transcriptase and Mixture of RNA Polymerase to RNA Polymerase List -- And (ii) The solution containing the stabilizing agent chosen from the group which changes from the mixture of a nonreducible disaccharide and a polyvinyl pyrrolidone to a nonreducible disaccharide and polyvinyl-pyrrolidone list is offered. A (b) this solution is frozen. The above-mentioned approach including generating the freeze-drying object which is made to sublimate the solvent part of the frozen (c) this solution by vacuous application, and contains this active oxygen constituent and this stabilizing agent by it.
2. Approach according to claim 1 said active oxygen constituent essentially consists of reverse transcriptase and mixture of RNA polymerase.
3. Approach according to claim 2 said stabilizing agent essentially consists of trehalose.
4. Approach according to claim 2 said stabilizing agent essentially consists of polyvinyl pyrrolidone.
5. Approach according to claim 2 of containing further deoxyribonucleotide triphosphoric acid with said solution sufficient when said desiccation powder is reconfigurated and it mixes with suitable nucleic-acid substrate and suitable reactant making possible both DNA polymerization and RNA transcripion, ribonucleotide triphosphoric acid, metal salt, and subfactor in single solution.
6. Approach according to claim 5 said solution contains further at least one kind of oligonucleotide magnification primer.
7. Method according to claim 5 of holding the capacity for said enzyme constituent to amplify target-nucleus acid, at least 70%, when said freeze-drying object is stored for two months at room temperature.
8. Method according to claim 5 of holding the capacity for said enzyme constituent to amplify target-nucleus acid, at least 70%, when said freeze-drying object is stored for two months at 35 degrees C.
9. Method according to claim 5 of holding the capacity for said enzyme constituent to amplify target-nucleus acid, at least 70%, when said freeze-drying object is stored for two months at 45 degrees C.
10. The method according to claim 5 of holding the capacity for said enzyme constituent to amplify a target-nucleus acid, at least 70%, when said freeze-drying object is stored for two months at 55 degrees C.
11. The constituent containing the freeze-dried reverse transcriptase.
12. The constituent containing the freeze-dried RNA polymerase.
13. It is Constituent for Magnification of Target-Nucleus Acid. In RNA-Dependent-DNA-Polymerase Activity of (a) Effective Dose, DNA-Dependent-DNA-Polymerase Activity, RNase H Activity and DNA-Dependent-RNA-Polymerase Activity, and Here RNA-dependent-DNA-

polymerase activity, DNA-dependent-DNA-polymerase activity, and RNase H activity are given with one kind or the separate enzyme beyond it. One kind or the stabilizing agent beyond it which has (b) freezing protection activity, (c) deoxyribonucleotide triphosphoric acid and ribonucleotide triphosphoric acid (d) metal salt -- and -- the single freeze-drying object which has the (e) reducing agent -- containing -- In here When reconfigurating this freeze-drying object by addition of an aquosity solvent, the solution obtained When this solution is incubated at sufficient temperature to add the single-stranded-RNA molecule which has a target nucleotide array area and one kind, or the suitable oligonucleotide primer beyond it to this solution, and promote this enzyme activity The above-mentioned constituent characterized by amplifying this RNA molecule.

14. The constituent according to claim 13 with which said one kind or suitable primer beyond it is contained in the freeze-drying object.

15. The constituent according to claim 13 with which said RNA-dependent-DNA-polymerase activity, DNA-dependent-DNA-polymerase activity, and RNase H activity are given with recombination retrovirus reverse transcriptase, and said DNA-dependent-RNA-polymerase activity is given by bacteriophage RNA polymerase.

16. The constituent according to claim 15 with which said reverse transcriptase originates in a MORONI murine leukemia virus.

17. The constituent according to claim 15 with which said RNA polymerase originates in T7 bacteriophage.

18. The constituent according to claim 13 chosen from the group to which said stabilizing agent changes from the mixture of a nonreducible disaccharide and a polyvinyl pyrrolidone to a nonreducible disaccharide and polyvinyl-pyrrolidone list.

19. The constituent containing the stabilizing agent chosen from the group which consists of a sucrose, trehalose, and a polyvinyl pyrrolidone according to claim 13.

20. The constituent according to claim 13 with which said stabilizing agent contains trehalose.

21. It is Constituent for Magnification of Target-Nucleus Acid. In RNA-Dependent-DNA-Polymerase Activity of (a) Effective Dose, DNA-Dependent-DNA-Polymerase Activity, RNase H Activity and DNA-Dependent-RNA-Polymerase Activity, and Here RNA-dependent-DNA-polymerase activity, DNA-dependent-DNA-polymerase activity, and RNase H activity are given with one kind or the separate enzyme beyond it. One kind or the stabilizing agent beyond it which has (b) freezing protection activity, (c) deoxyribonucleotide triphosphoric acid and ribonucleotide triphosphoric acid, (d) metal salt and the (e) reducing agent -- and -- the single freeze-drying object which has (f) buffer solution -- containing -- however, conditional [that this constituent does not contain a carboxylic acid] -- it is -- In here When reconfigurating this freeze-drying object by addition of an aquosity solvent, the solution obtained When this solution is incubated at sufficient temperature to add the single-stranded-RNA molecule which has a target nucleotide array area and one kind, or the suitable oligonucleotide primer beyond it to this solution, and promote this enzyme activity The above-mentioned constituent characterized by amplifying this RNA molecule.

22. The constituent according to claim 21 with which said RNA-dependent-DNA-polymerase activity, DNA-dependent-DNA-polymerase activity, and RNase H activity are given with recombination retrovirus reverse transcriptase, and said DNA-dependent-RNA-polymerase activity is given by bacteriophage RNA polymerase.

23. The constituent according to claim 22 with which said reverse transcriptase originates in a MORONI murine leukemia virus.

24. The constituent according to claim 22 with which said RNA polymerase originates in T7 bacteriophage.
25. The constituent according to claim 21 chosen from the group to which said stabilizing agent changes from the mixture of a nonreducible disaccharide and a polyvinyl pyrrolidone to a nonreducible disaccharide and a polyvinyl-pyrrolidone list.
26. The constituent according to claim 22 chosen from the group to which said stabilizing agent changes from a nonreducible disaccharide and a polyvinyl pyrrolidone.
27. The constituent according to claim 22 with which said stabilizing agent contains trehalose.
28. the freezing protection stabilizing agent chosen from the group which consists of trehalose and a polyvinyl pyrrolidone in the reverse transcriptase and RNA polymerase which are the kit for magnification of a target-nucleus acid, and were together put in the single freeze-drying compound -- together -- containing -- here -- setting -- the time of addition of rehydration of this freeze-drying compound, and the target-nucleus acid under existence of an oligonucleotide primer -- some -- or the above-mentioned kit with which these all target-nucleus acids are amplified.
29. The kit according to claim 28 with which said freeze-drying compound contains a metal salt and nucleotide triphosphoric acid further.
30. The kit according to claim 28 which contains further at least one kind of oligonucleotide magnification primer.
31. The kit according to claim 28 with which said stabilizing agent contains trehalose.
32. The kit according to claim 28 with which said stabilizing agent contains a polyvinyl pyrrolidone.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
Field of enzyme constituent invention which was [for nucleic-acid magnification] stable This invention relates to the field of molecular biology, and the living body constituent stabilized [which stabilized and was nucleic-acid-amplified] generally. Especially this invention relates to the stable freeze-drying enzyme constituent containing one kind or the nucleic-acid polymerase beyond it.
Background of invention A deoxyribonucleic acid (DNA) and a ribonucleic acid (RNA) are large-sized straight chain-like macromolecules which consist of the nucleotide subunit which carried out covalent bond. DNA is usually found out in the form of the "double strand" which two DNA strands have combined in the antiparallelism format by hydrogen bond. RNA usually exists naturally as one polynucleotide chain. A nucleotide is a molecule which has sugar (or deoxyribose or ribose) and a nitrogen-containing base part, and by the phosphodiester bond, it joins together mutually and it has usually become a nucleic acid. Five kinds of usual nitrogen-containing bases exist. Three kinds are seen also by DNA or RNA, and these are an adenine (A), a guanine (G), and a cytosine (C). Other two are a thymine (T) and a uracil (U), and they are peculiar to DNA and RNA respectively.
the greater part of all living thing genetic information (all -- coming out -- ****** there is nothing) -- the form of DNA or RNA -- the generation of one generation to a degree -- ** -- it is told. This information is told in the array of the nucleotide which met, one nucleic-acid chain, i.e., a "chain", which constitutes a genetic code. or [furthermore, / that the nitrogen-containing base

of a nucleic-acid chain is the same respectively] -- or it has the capacity which carries out hydrogen bond to other different nitrogen-containing bases beyond one kind or it of a nucleic-acid chain specifically. Therefore, A carries out hydrogen bond to T (or U) under the usual condition, and C carries out hydrogen bond to G, and this specific hydrogen bond is called a base pairing. In the case of double stranded DNA, each of the two chains consist of one nucleotide chain, and most or all of these nucleotides is carrying out the base pairing to another chain. In such a case, the sequence of the nucleotide on one DNA strand determines the sequence of the nucleotide on another DNA strand. Two nucleic-acid chains which are "mirror images" mutually are said to be completely complementary by this method.

A nucleic acid is compounded by in vivo one by the mechanism using the fact that each nucleic-acid chain specifies the sequence of the nucleotide of a completely complementary chain, and if a desired nucleic acid is RNA and this will be DNA, it will not change the nucleic acid used as mold regardless of whether it is RNA or it is DNA. Most peculiar mechanisms for DNA replication need use of DNA polymerase, in order to add a nucleotide to a target serially to 3' hydroxyl of the polynucleotide primer which carried out hydrogen bond to the mold nucleic-acid chain. The nucleotide newly added is chosen by DNA polymerase based on those capacity that carries out involution to the nucleotide to which a template strand corresponds. This process in which NUKURENUOCHIDO is added to the end of a primer is sometimes called primer expanding.

Unlike DNA synthesis, RNA biosynthesis does not usually need existence of a polynucleotide primer. Rather, RNA biosynthesis is usually carried by the RNA polymerase which recognizes the specific nucleotide sequence beyond one piece or it of nucleic-acid mold. The mold field which is called a promotor and which RNA polymerase combines is usually a double strand. After combining with a promotor, RNA polymerase compounds the complementary polyribonucleotide chain which carried out covalent bond for a template strand to "read" and its mold. The RNA polymerase originating in a different living thing recognizes a different promotor array preferentially.

DNA and an RNA polymerase enzyme have been refined from the living thing from which a large number differed. Generally some of these enzymes, such as Klenow fragment of Escherichia coli (E. coli) DNA polymeraseI and DNA polymerase I and various RNA polymerase, are used by in vitro one as a means of molecular biology and nucleic-acid biochemistry research. Generally, please refer to Sambrook et al. (Sambrook) and Molecular Cloning:A Laboratory Manual (the 2nd edition, cold spring harbor press 1989 (Cold Spring Harbor Press)).

Another use of nucleic-acid polymerase started with the advent of the various approaches of nucleic-acid magnification, such as polymerase chain reaction (PCR). For example, please refer to Mullis et al. (Mullis), a U.S. Pat. No. 4,683,195 specification, a 4,683,202 specification, and a 4,800,159 specification. In the easiest form of PCR, two kinds of oligonucleotide primers which are respectively complementary primers are compounded by the field of the target-nucleus acid located in 3' side about the target-nucleus acid among target nucleotide array areas. Each Plummer is complementary to one side of two complementary nucleic-acid chains, and the target field includes the nucleotide array area which includes the nucleic-acid chain of both double strand target-nucleus acids. Hydrogen bond ("hybridization") of these primers is carried out to a substrate, and when DNA polymerase is added to a reaction mixture together with nucleotide triphosphoric acid, each primer which carried out hybridization is elongated in the 5'->3' direction with an enzyme. Next, a reaction mixture is heated, a primer expanding product:mold

hybrid is dissolved, temperature is reduced, a primer / target hybridization is made possible once again, and the DNA polymerase which added more DNA polymerase and deactivated in the elevated-temperature phase is permuted. the number ******** of cycles of a request of the process -- by things, the amount of the nucleic acid which has a target nucleotide sequence increases exponentially. Furthermore, the heat stability DNA polymerase originating in the Thermus AKUA tex (Thermus aquaticus) is used with the sufficient result by the PCR method, and decreased the need of repeating an expensive enzyme so much and adding it recently. Since Taq polymerase is equal to inactivation at 90-95 degrees C, it abolishes the need of adding an enzyme repeatedly after chain separation of each time.

Other approaches of nucleic-acid magnification that RNA transcripion is used as a single step of a magnification process are devised. After such one approach's making one of the primers used at an PCR reaction include a promotor array and making it amplify by the PCR method next, it works by using double stranded DNA as mold of an imprint of the single stranded RNA by DNA dependent RNA polymerase. For example, please refer to MURAKAWA et al. (Murakawa) and DNA 7:287-295 (1988).

Other amplifying methods use many cycles of RNA dependency DNA synthesis and an imprint for making DNA or an RNA target amplify. For example, Berg et al. (Burg), and WO 89/No. 1050; gin JIERASU et al. (Gingeras) and WO 88/No. (sometimes) 10315 ; KASHIAN (Kacian) and FURUTSU (Fultz) which are called an imprint multiplier system or TAS, Please refer to EPO public presentation [No. (it has the same ownership person as this application) 408,295]; application [Davie (Davey) and Marek (Malek), and the EPO application 88113948.No. 9]; Marek et al. and WO 91/No. 02818. The reverse transcriptase (RT) which is an enzyme which can use RNA or DNA as mold of composition of a complementary DNA chain is used for these approaches. Some of these approaches use cellularity RNase H activity as an indispensable component again. The retrovirus reverse transcriptase of most, such as that in which a code is carried out by a MORONI murine leukemia virus (MMLV) and the Tori myeloblastosis virus (AMV), has RNase H activity in RNA dependent DNA polymerase, DNA-dependent-DNA-polymerase activity, and a list. Since RNase H activity destroys alternatively the RNA chain of a RNA:DNA hybrid nucleic-acid molecule, it advances a magnification reaction, without needing temperature circulation.

nucleic-acid magnification is peculiar in various setup -- again -- ** -- it is a still more general means for specific discernment of a characteristic nucleic-acid segment, and/or magnification. Therefore, nucleic-acid magnification is used for a food agricultural trial, medical diagnosis, human gene analysis and counseling, and an archaeology list by criminal discussion. Since all of these approaches use an enzyme, the approach of reaching and storing packed and conveyed of manufacturing a very activity enzyme so much serves as a very important trouble in manufacture of the enzyme for nucleic-acid magnification, and a kit, marketing, and sale. Concretely, the approach and preparation for storing reverse transcriptase and the activity preparation of RNA polymerase about the approach using the magnification based on an imprint which can be permitted commercially are indispensable to the manufacture and marketing which were successful about the kit for nucleic-acid magnification.

The general method of stabilizing reverse transcriptase and an RNA polymerase enzyme (a majority of other enzymes used for a list by molecular biology research) depends the liquid preparation of each enzyme in the solution containing reducing agents, such as 50% (v/v) glycerol and dithiothreitol (DTT), or beta-mercaptoethanol (betaME), on storing at -20 degrees C. This approach saves some activity of an enzyme, without monthly [most] losing activity. When

by contrast it is a room temperature or stores an enzyme at 4 degrees C, enzyme activity is lost easily. As for these preparations, it is common that even an end user is conveyed in dry ice from an enzyme feeder, and enzyme activity decreases 30% or more than it generally for freeze thawing of an enzyme preparation in the case of such transportation. These enzymes are blended separately and supplied.

The method of storing and conveying reverse transcriptase and RNA polymerase, without needing refrigeration will abolish the need for refrigeration transportation of dry ice, a wet pack, a dry pack, or a SUCHIRO form transport vessel and/or cold preservation. Since the production cost related to these methods of maintaining enzyme activity is unnecessary for such an approach again, cost effectiveness is considered to be better. The enzyme storage condition which makes it possible to bear limited elevated-temperature exposure with an enzyme preparation will lose reduction of the enzyme activity which may happen, when it is on a loading dock or all over a truck while the enzyme preparation conveys. Probably, such an approach should be extremely reproducible. Furthermore, probably, such a preparation will be convenient by it being economical and using it by manufacturing, when provided in the form where the enzyme suited those purpose applications (inside of the compound containing most of required subfactors of arbitration, and most of [all or] etc.) in the single container.

Freeze drying (lyophilization) has been used for saving food, a biomembrane, all cells (for example, referring to American Society for Microbiology and Manual of Methods for General Bacteriology 210-217 (1981)), and a biopolymer including an enzyme. Freeze drying needs to remove water from a freezing sample by sublimation under reduced pressure. Sublimation is the process in which a solid-state is evaporated, without passing through a liquid condition.

The theoretical aspect of affairs of freeze drying is complicated. When biological substances, such as protein, are in a water solution, it is useful to it being thought that that molecule is surrounded with the hydration shell containing a water molecule, and this hydration shell stabilizing protein, and maintaining that activity. When water is removed, the reactant radical of the protein usually hidden by ****** becomes free, reacts mutually, and newly forms irreversible association essentially by it.

These association may distort proteinic natural conformation. Furthermore, under un-existing [of water], new canal / hydrophilic interaction may happen, and this may also distort proteinic conformation. Since the three-dimensions conformation of much protein gives biological activity, the distortion of the conformation may change biological activity at the time of desiccation. By the same mechanism, proteinic bridge formation and proteinic condensation may take place.

It is useful to lessening conformation skewness by desiccation to make it freeze, before drying a protein sample. The lowered initial temperature is useful to maintaining the reaction which is not [between amino acid reactivity radicals] desirable to the minimum by taking the energy of a reactant. The protein in a freezing condition has the inclination no conformation change on the whole three-dimensional not much freely than the case of a solution to be almost in coincidence. However, the freeze-drying object dried completely has an inclination shorter, "preservation, i.e., storage life,", than the case of the freeze-drying object which still contains the water of a low percentage and which was dried imperfectly. The inactivation study reaction which cannot occur may advance in the place where such a freeze-drying object dried imperfectly often needs to be stored at the temperature of about 4-10 degrees C or less at, and water does not exist in addition. Therefore, although the shelf life of the biological activity protein freeze-dried by much imperfect desiccation is longer than what is dried completely, in order to maintain activity, in addition, it is required for refrigerating a preparation. But [even if so] the activity of such a

preparation decreases comparatively for a short period of time. Furthermore, whether the preparation is dried completely does not concern and have the enzyme of some, such as phosphofructokinase, and it deactivates completely after freeze drying under un-existing [of cryoprotectant].
For example, please refer to carpenters (Carpenter) and Cryobiology 25:372-376 (1988).
The vocabulary the "cryoprotectant" used in this specification means the compound or constituent which tends to protect the activity in the case of freezing of a biological active substance, desiccation, and/or reconstruction of the dry substance.
The vocabulary a "stabilizing agent" means the matter which bars or delays a reduction of the biological activity of the matter with the passage of time as compared with the case where the matter is stored under un-existing [of a stabilizing agent], when added to a biological active substance.
When drying a biomaterial including specific protein, in order to use as an excipient which is useful to saving biological activity, various cryoprotectant additives have been used or proposed. KUREGGU et al. (Clegg) and Cryobiology 19:106-316 (1982) have studied the role of the glycerol in the capacity of the cyst of marine shrimp ARUTEMIA (Artemia) in the condition that after the dry maintenance of idle boiler may survive, and/or trehalose.
Carpenters and Cryobiology 24:455-464 (1987) have reported that the role to which a disaccharide maltose, a sucrose, a lactose, and trehalose make stabilization of phosphofructokinase activity increase in the purification enzyme preparation by which the air-drying was carried out can be played. The EPO public presentation 0431882ANo. 2 is indicating the preparation by which the purification alkaline phosphatase freeze-dried under existence of a mannitol or a lactose was stabilized, after being derivatized. The EPO public presentation 0091258ANo. 2 is indicating the method of stabilizing a tumor necrosis factor (TNF) by the storage or freeze drying of purification protein under existence of stabilization protein, such as a human serum albumin, gelatin, a human gamma globulin, or salmon protamine sulfate. The U.S. Pat. No. 4,451,569 specification is indicating use of the pentose which stabilizes the activity of purification glutathione peroxidase, sugar-alcohol, and some disaccharide. The stable constituent can be stored at the temperature of less than 20 degrees C, after being freeze-dried. The EPO public presentation 0448146ANo. 1 is examining the stable freeze-drying gonadotropin preparation containing a dicarboxylic acid salt. The preparation can contain disaccharides, such as a sucrose or trehalose, further. ROSA (Roser), Biopharm, and 47-53 (September, 1991) are examining saving the biological activity of the various biomolecules dried with ambient temperature using trehalose. the [PCT public presentation] -- WO 87/No. 00196 has reported stabilization of the monoclonal antibody by the air-drying under existence of trehalose, and cow intestines alkaline phosphatase. the [PCT public presentation] -- the [WO 89/No. 00012 and / this] -- WO 89/No. 06542 is considering use of the trehalose for saving some food and antigenic [of valid virion]. The EPO public presentation 02270799ANo. 1 has reported stabilization of the recombination beta interferon in the compound containing stabilizing agents, such as a surfactant or glycerol. The constituent can contain further the various sugar which includes a sucrose and trehalose as an additional stabilizing agent, ** alcohol, and protein, and glucose is among these the most desirable.
When some of these additives are mostly stored by dehydration in ambient temperature, it turns out that some shelf lives of a biological active substance are extended to the moon or more than it. However, the effectiveness, the compatibility, or the advantage of the concrete additive expected depends on the chemical composition of the biological active substance called for so

that it may stabilize, and it can mention secondary [the], Miyoshi, and quaternary structure to the amino acid sequence of the protein, and a list, without being limited as these factors in the case of protein. Therefore, it cannot expect deductively whether it works so that a specific constituent may save the biological activity of a specific biological active substance.
Furthermore, when making protein freeze-dry, the factor of the addition which included the die length of a freeze-drying procedure in a buffer-solution presentation, freezing velocity, the amount of negative pressure, the initial temperature of freeze drying, working temperature, and a terminal temperature list is important for determining the stability and the shelf life of activity protein, without being limited to these.
It is known that some protein has much enzyme activity. For example, retrovirus reverse transcriptase (MMLV-RT), such as an enzyme originating in a MORONI murine leukemia virus, has DNA-dependent-DNA-polymerase activity, RNA-dependent-DNA-polymerase activity, and RNase H activity. Although such activity is included in the same enzyme, the conditions for saving any one of the activity of such in a desiccation preparation do not promise to be saved under the same conditions for both both [remaining / one remaining or].
furthermore, like the case of KASHIAN and FURUTSU, and the nucleic-acid multiplier system based on the imprint of the above (it has the same ownership person as this application, and used into this specification) Balance of the phase contrast activity of three kinds of activity of reverse transcriptase When required of the application of specification [saying / that after reconstruction is in the same condition as balance of such activity before desiccation], specific Conservation Act makes delicate balance of such enzyme activity lose, and may make this kind of use make that enzyme unsuitable by it. For example, when more RNase activity of an enzyme than RNA-dependent-DNA-polymerase activity is saved, a RNA:DNA initiation complex may be disassembled before being able to start DNA synthesis.
Since the effectiveness or advantage of the effective in the mothball of a certain given enzyme activity freezing protection constituent given at the time of being applied to another enzyme activity is not clear, various enzymes often need an argument which is completely different about activity stabilization.
As a result, all of the freeze-drying enzyme preparations manufactured commercially or most contains only one kind of dried enzyme in the compound by which job order production was carried out so that the activity of the specific enzyme might be saved.
Outline of invention This invention relates to the constituent and kit containing the desiccation compound of the reverse transcriptase and RNA polymerase which can carry out the long term storage of the enzyme activity with ambient temperature, without decreasing substantially. Preferably, this compound contains the preparation of retrovirus reverse transcriptase and/or bacteriophage RNA polymerase. Furthermore, this compound is the reverse transcriptase (MMLV-RT) and bacteriophage T7 which originate in a freezing protection excipient at a MORONI murine leukemia virus preferably. RNA polymerase is included. In addition, this invention is MMLV-RT and T7 in one kind or the freezing protection excipient beyond it much more preferably. It is related with the single container containing the desiccation compound containing both RNA polymerase. This invention is MMLV-RT and T7 most preferably. RNA polymerase, One kind or the freezing protection excipient beyond it containing either trehalose or a polyvinyl pyrrolidone (PAP) and both, It is the single container which contains the desiccation compound containing a metal ion required for said enzyme activity, and a subfactor in nucleotide triphosphoric acid and a list. It is related with the above-mentioned single container which is a form with the conveniently sufficient cost effectiveness to nucleic-acid magnification,

without this compound needing too much handling in the case of addition of the suitable primer beyond a target-nucleus acid and one kind, or it in the reconstruction list of the stable freeze-drying object. By the case, such a compound may contain the primer for initiation of nucleic acid biosynthesis. Finally, this invention relates to the manufacture approach of the above-mentioned desiccation compound, and operation.
reverse transcriptase and an RNA polymerase enzyme -- the [Berg et al., above-mentioned; gin JIERASU et al., above-mentioned (sometimes called imprint multiplier system or TAS); KASHIAN and FURUTSU, and; Davie and above-mentioned Marek and above-mentioned EPO application 88113948.No. 9; and Marek et al., and / PCT public presentation] -- in the imprint medium nature nucleic-acid amplifying method which was indicated by WO 91/No. 02818, it is the important matter. Such approaches are requirements with manufacture of the product with which the stability of a magnification reagent with the passage of time uses nucleic-acid magnification, marketing, and the costs of use in fields, such as legal medicine and medical diagnosis, it is more important still, and important there.
The applicant discovered the approach and desiccation compound for preservation of the DNA dependent DNA polymerase of reverse transcriptase, RNA dependent DNA polymerase, and RNase H activity. It was discovered that this approach and the compound fit preservation of RNA polymerase activity. Furthermore, the applicant discovered stabilizing as a desiccation compound in a single container, and it being saved, and getting, without almost losing four kinds of activity [each] over the period when after both enzymes and an incubation hot [four kinds] in all enzyme activity and prolonged is remarkable also unexpectedly.
One mode of this approach is a nonreducible disaccharide (preferably) about activity purification reverse transcriptase. Together with the freezing protection excipient containing a sucrose, trehalose, or a polyvinyl pyrrolidone (PAP) Or although the solution containing reverse transcriptase and this cryoprotectant is not necessarily limited, after drying an enzyme by approaches, such as freeze drying It includes providing together with the mixture of these compounds of an amount effective in acting as matter which protects and saves the DNA dependent DNA polymerase, RNA dependent DNA polymerase, and RNase H activity of reverse transcriptase.
the second voice -- like -- setting -- purification RNA polymerase [activity / this invention] -- desirable -- T7 It is characterized by the approach of stabilizing and saving RNA polymerase by stable dehydration substantially exceeding 90 days at a room temperature. A metal salt; nonreducible disaccharide in which RNA polymerase contains Mg++ or Zn++ in this mode, one kind preferably chosen from the group which consists of trehalose and a polyvinyl pyrrolidone (PAP), or the protection stabilizing agent beyond it; a list dries under existence of reducing agents, such as n-acetyl-L-cysteine (NALC). Although it does not desire to be restricted by the theory, the applicant thinks that it is useful to a reducing agent barring deactivation of the enzyme by oxidation of the cysteine residue of the arbitration which exists in an enzyme. In this mode, RNA polymerase holds at least 70% of the activity of the origin of it for that dehydration compound after exposure for at least 61 days at 35 degrees C for at least 30 days to the temperature of 45 degrees C preferably.
In another mode, this invention is characterized by reverse transcriptase (preferably MMLV-RT), RNA polymerase (preferably T7 RNA polymerase), the freezing protection excipient (preferably trehalose and/or a polyvinyl pyrrolidone) of an amount effective in saving the enzyme activity of a desiccation enzyme, nucleotide triphosphoric acid, the required subfactor, the oligonucleotide primer of arbitration and the reducing agent, and the single desiccation compound that contains

the mixture of thiol compounds preferably.
Furthermore, in another mode, this invention contains the component of the kit for one kind or the kind beyond it of the inside for magnification of the nucleic acid belonging to one kind or the phylogeny classification beyond it of a living thing, and specific discernment (for example, a group), one kind in **, or specific detection of the group beyond it. This invention offers the desiccation compound which contains reverse transcriptase, RNA polymerase, ribonucleotide triphosphoric acid, deoxyribonucleotide triphosphoric acid, zinc and/or magnesium salt, and a reducing agent in a single container and which can be reconfigurated. A magnification primer and a reconstruction water solution can be given as still more nearly another component beyond one kind or it of a kit. Or a magnification primer can be contained in a desiccation compound. A target sequence specific nucleic-acid hybridization assay probe and the desirable non-indicator helper oligonucleotide of arbitration can be contained in a desiccation compound, or can be offered with another reagent. After adding reconstruction and the oligonucleotide primer of a desiccation compound (when it has not already existed), mixture is contacted in an imperfect or perfect single strand target-nucleus acid. The reaction will advance in the case of the incubation of the reaction mixture in sufficient temperature for nucleic-acid magnification, when a target-nucleus acid has one piece, or two or more primers (or primer part of a promotor-primer (one piece or plurality)) and a complementary nucleotide sequence.
In another mode, this invention contains reverse transcriptase (preferably MMLV-RT), RNA polymerase (preferably T7 RNA polymerase), a freezing protection excipient, nucleotide triphosphoric acid, a required subfactor, and the single freeze-drying object containing the combination of a reducing agent which has a thiol group preferably. without it is conveyed, and this freeze-drying object is stored, without needing refrigeration and it decreases Japanese lacquer and enzyme activity intentionally -- temporary elevated-temperature exposure -- for example, it can bear in 30 days at 55 degrees C, without being limited.
"Nucleotide triphosphoric acid" means Libor who can serve as a substrate of RNA polymerase and DNA polymerase (preferably reverse transcriptase), respectively or deoxyribonucleotide triphosphoric acid, and those derivatives. As such a derivative, the nucleotide which has the methyl (or other alkyls) and/or the sulfur radical which were included by the nitrogen-containing base (usually an adenine, a thymine or a uracil, a cytosine, and a guanine), the ribose, the deoxyribose part, or the phosphoric-acid radical can be mentioned, without being limited.
A "nucleotide" means the nucleic-acid subunit containing a part for one nitrogen-containing base (usually an adenine, a thymine or a uracil, a cytosine, and a guanine) and a sugar part (a ribose or deoxyribose), and a phosphoric-acid radical. The vocabulary used in this specification means both nucleotide subunit in which Libor or deoxyribonucleotide triphosphoric acid which is not incorporated and the oligonucleotide, or the nucleic-acid chain carried out covalent bond according to the context used.
Detailed description This invention is the approach of stabilizing the enzyme activity of DNA polymerase and an RNA polymerase enzyme, and relates to the above-mentioned approach by removing a solvent from the solution which contains more than one kind or it of these enzymes under existence of cryoprotectant or stabilization "an extending agent." As such cryoprotectant, there is a water-soluble polymer which has a saccharide, a positivity available although hydrogen bond is especially carried out to a nonreducible disaccharide and an enzyme, and/or an electronegative radical. Especially desirable cryoprotectant is the polyvinyl pyrrolidone (PAP) of a polymer at the sucrose and trehalose list of a disaccharide.
This invention relates to the stable constituent containing the mixture which contains further both

the DNA polymerase by which the dry maintenance of idle boiler was carried out, the RNA polymerase by which the dry maintenance of idle boiler was carried out or DNA polymerase, and RNA polymerase and by which the dry maintenance of idle boiler was carried out. The desirable enzymes containing these constituents are reverse transcriptase and bacteriophage RNA polymerase, and;, especially a desirable enzyme are MORONI murine leukemia virus origin retrovirus reverse transcriptase and bacteriophage T7 origin RNA polymerase.
The desirable approach of carrying out the dry maintenance of idle boiler of the DNA polymerase and RNA polymerase of this invention is based on freeze drying. By this approach, a vacuum is applied to the enzyme solution which was made to freeze an enzyme content solution and was frozen, and sublimation removes a solvent from a preparation, and it leaves a solute.
Further, this invention is a constituent for the duplicate of one kind or the specific nucleic-acid array beyond it, and is characterized by DNA polymerase (preferably reverse transcriptase), RNA polymerase, nucleotide triphosphoric acid, and the above-mentioned constituent containing the preparation to which the dry maintenance of idle boiler of the subfactor required for enzyme activity was carried out. The preparation by which the dry maintenance of idle boiler was carried out can contain further the magnification primer and/or hybridization assay probe, and helper of **** of a target nucleotide sequence. [of a specific duplicate] Preferably, a dry-maintenance-of-idle-boiler constituent is manufactured with freeze drying.
The constituent of this invention is stable for a long period of time, also when stored at an elevated temperature even if.
Such a constituent is useful in the transportation and storage of the kit for nucleic-acid magnification which follow and contain the commercial preparations of these enzymes, and these enzymes.
Example The following examples do not illustrate various embodiments of this invention desirable now, and not restricting the range anyway will be understood. It is not shown that other embodiments of effective this invention may not exist by corresponding by this invention attaining one or the purpose beyond it called for also for the indication of an embodiment.
example 1: -- freeze drying of reverse transcriptase and RNA polymerase it was discovered by the Escherichia coli (E. coli) strain 1200, and the reverse transcriptase used in this example and the following examples was refined from the cell paste -- rearranging -- MORONI murine leukemia virus reverse transcriptase -- or it received from united States biochemicals (United States Biochemicals), Cleveland, and Ohio -- it was an available purification MMLV-RT preparation commercially. An enzyme preparation is 20 - 50mM tris. - It is HCl (pH7.5) and 0.1M. NaCl, 0.1mM ethylenediaminetetraacetic acid (EDTA), 1.0mM dithiothreitol (DTT), 0.01%(v/v) TERGITOL NP-40 (TERGITOL NP) TRTTON 0.1% (v/v), or it is the trademark of Union Carbide Chemicals - and - Plus Tex Company, Incorporated (Union Carbide Chemicals and Plastics Co., Tnc.) It was stored at -20 degrees C into the buffer solution for storage containing X-100 (TRITON is the trademark of Union Carbide Chemicals - and - Plus Tex Company, Incorporated), and 50% (v/v) glycerol. Purification T7 RNA polymerase came to hand from EPISENTORU Technologies (Epicentre Technologies), Madison, and WI. Before dialysis, they are 50% (v/v) glycerol and 50mM tris about an enzyme. - HCl (pH7.5), 0.1M NaCl, 1.0mM DTT, 0.1mM It stored in EDTA and 0.1%(v/v) TRITON X-100. This enzyme was also stored at -20 degrees C before dialysis.
Three kinds of enzyme preparations were dialyzed to freeze drying. The first preparation is 20mM. HEPES ([2-hydroxyethyl] piperazine - N' - [2-ethane sulfonic acid]) (pH7.5)0.1M NaCl, 0.1mM EDTA, 2mM MMLV-RT diluted in the buffer solution containing

NALC, a 0.1mM zinc sulfate, 0.2M trehalose, and water 324,012 units were included. The last capacity was 720microl. About this, it is this buffer solution (trehalose buffer solution (Trehalose Buffer)).
It dialyzed at 4 degrees C to 250ml for 6 hours. permeable membrane -- 2% (w/v) sodium bicarbonate and 10mM(s) the inside of EDTA (pH8.0) -- next, 10mM It was prepared by boiling the inside of EDTA (pH8.0), and the last for 10 minutes in deionized water, respectively. Next, the film was fully washed by deionized water before use. The buffer solution for dialysis was exchanged for the buffer solution with fresh tales doses, and dialysis was continued for further 10 hours. The buffer solutions were exchanged again and it continued for further 3 hours. The last capacity was 655microl.
The second preparation is T7 in 720microl. RNA polymerase 144,000 unit was included. To the trehalose buffer solution, it is the same plan as a reverse transcriptase preparation, and this was dialyzed with tales doses. The last capacity was 1270microl.
The third preparation was taken as last capacity 1440microl combining reverse transcriptase 324,012 unit and RNA polymerase 144,000 unit including both reverse transcriptase and RNA polymerase. This was dialyzed in the same plan as other two preparations to the trehalose buffer solution of 3 equivalence. The last capacity of dialysing fluid was 1975microl.
Each preparation was divided into the vial as an aliquot of 12 division into equal parts after dialysis. Each vial is reverse transcriptase 27,000 unit and T7. RNA polymerase 12,000 unit or both enzymes were included in these amount. Those vials were put into BIRUTISU (Virtis) mold lyophilizer 101-solvent refined coal equipped with the FCP-III control system which can be programmed. The vial was cooled to -40 degrees C in about 5 minutes. Freeze drying was started by reducing a pressure to -180torr, and the vacuum was kept constant among the freeze-drying protocol. Next, temperature was linearly raised during the following 2 hours to -10 degrees C, and it maintained at this temperature for the following 6 hours. Next, the temperature was linearly raised during the following 1 hour to 10 degrees C, and it maintained at 10 degrees C for 4 hours. The temperature was raised again linearly over [of a degree] 30 minutes to 25 degrees C, and it maintained at 25 degrees C for the following 10.5 hours. Next, the pressure was returned to the atmospheric pressure by installation of desiccation nitrogen, and before taking out a vial from a lyophilizer, they were sealed under nitrogen. Next, the vial was stored for 22 days at 25 degrees C.
The freeze-dried enzyme preparation was reconfigurated after the storage time in buffer-solution (Reconstitution Buffer) for reconstruction (0.01%(v/v) TRITON X-100, 41.6mM MgCl2, 1mM ZnC2H3O2 or 10% (v/v) glycerol, 0.3% (v/v) ethanol, 0.02% (w/v) methylparaben, and 0.01% (w/v) propylparaben), and it authorized about those capacity that supports nucleic-acid magnification.
A reaction mixture with a full capacity [l] of 90micro is 50mM tris. - HCl (pH8.0), 17.5mM(s), 2mM spermidine, 25mM KCl, dATP of two mM(s) each, dCTP, dTTP and dGTP, 2.5mM CPT and UTP, 6.5mM(s) ATP and GTP, 5mM 675microg [/ml] solution 0.44microl of a promotor-primer (array number: 1) which has a complementary target joint field to the field of one chain of DTT and bacteriophage T7 gene 10, It was prepared including 451microg [/ml] solution 0.3microl and T7 gene 10 target-nucleus acid 100 copy and water of the primer (array number: 2) which has a complementary target joint field to another chain of bacteriophage T7 gene 10. The T7 gene 10RNA target was (+) sense imprint object of the plasmid origin T7 gene 10 restriction fragment originating in plasmid pGEMEX-1 (a pro megger corporation (Promega Corporation), Madison, WI). The purification RNA transcript existed by the concentration of

0.61 picomoles / mul. Target-nucleus acid 100 copy was added to each test tube. Multistory [of the 200micro of the mineral oil l] was further carried out to each test tube, and evaporation of the sample under assay was prevented.
All test tubes incubate for 5 minutes at 95 degrees C, and although it cooled to the room temperature before adding the enzyme reconfigurated as mentioned above, this process is unnecessary, when a target-nucleus acid is RNA or it is not double stranded DNA but a single stranded DNA, and an initial heating process is useful to dissolving one field of the RNA intramolecular hydrogen bond. next, the test tube containing the enzyme preparation freeze-dried separately -- T7 RNA polymerase 400 unit and freeze-drying MMLV-RT 600 units -- or 10micro of solutions l containing 900 units was put in. T7 by which coincidence freeze drying was carried out RNA polymerase and MMLV-RT existed by the concentration of 400 units, and 900 units / 10microl, respectively. The test tube was incubated at 37 degrees C for 3 hours.
Although the amount of the magnification nucleic acid produced during the reaction was measured by Arnold (Arnold) and Nelson (Nelson), and the U.S. Pat. No. 5,283,174 specification (it has the same ownership person as this application, and used into this specification) using homogeneity protection assay of a publication Probably, it will be clear to this contractor that many other assay systems and approaches of detecting a nucleic-acid target, such as to be because for a radioactive indicator probe to be used, are available in the technical field concerned.
A magnification reaction is a 200mM succinic-acid lithium (pH5.2) and 17% (w/v).
Lauryl lithium sulfate, 3mM EDTA (ethylenediaminetetraacetic acid) and 3mM(s) 100micro of hybridization buffer solutions l which contain a complementary acridinium ester indicator probe (array number: 3) in EGTA ([ethylene bis(oxy-ECHIRENITORIRO)] -4 acetic acid) and T7 gene 10 RNA transcript was ended by adding to each test tube. The test tube was incubated for 20 minutes at 60 degrees C. About the acridinium ester combined with the probe which has not carried out hybridization, they are 182mM(s) of 300microl. It hydrolyzed by adding NaOH, a 600mM boric acid, and 1%(v/v) TRITON X-100, and the test tube was incubated for 5 minutes at 60 degrees C. The remaining chemiluminescence is 0.4Ns. HNO3 Naka 1%(v/v) H2O2 It is 1M immediately after adding 200microl. When NaOH (200microl) was added immediately and a solution was alkalized, it was measured in RUMINO meter. A result is a relative light unit (RLU).
Although come out and reported, this is a number of a photon of scales emitted with the chemiluminescence indicator. A result is shown in the following table 1.

表１

２５℃で２２日間貯蔵された凍結乾燥酵素と非凍結乾燥酵素との比較				
	ＲＮＡ標的		負対照	
	MMLV-RT 600 単位および T7ポリメラーゼ 400 単位	MMLV-RT 900 単位および T7ポリメラーゼ 400 単位	MMLV-RT 600 単位および T7ポリメラーゼ 400 単位	MMLV-RT 900 単位および T7ポリメラーゼ 400 単位
液体 MMLV-RT および液体 T7 RNA ポリメラーゼ	321329	428872	1868	5630
凍結乾燥 MMLV-RT および液体 T7 RNA ポリメラーゼ	301253	463561	1681	1684
液体 MMLV-RT および凍結乾燥 T7 RNA ポリメラーゼ	549204	343582	1366	1545
凍結乾燥 MMLV-RT および凍結乾燥 T7 RNA ポリメラーゼ (別々に凍結乾燥)	415080	493779	1352	1374
同時凍結乾燥の MMLV-RT および T7 RNA ポリメラーゼ	677531 (MMLV-RT 900 単位)	654359	1376 (MMLV-RT 900 単位)	1296

MMLV-RT by which coincidence freeze drying of these results was carried out, and T7 the reaction mixture with which RNA polymerase combined the enzyme preparation with the liquid enzyme preparation of another enzyme -- or having caused RNA gene 10 label-magnification more effectively than the case where it is the reaction mixture with which both enzymes are not freeze-dried is shown. As compared with a liquid enzyme, there was no significant reduction in any capacity of the freeze-drying enzyme preparation which carries out the catalyst of the

magnification. therefore, a result -- each enzyme -- independent -- or it is proved further that storage by the dryness under existence of trehalose is effectively stable, and it gets by it together. The nucleic-acid magnification under these conditions shows effectively that it is maintained by the condition that activity harmonized by method which promotes the thing of reverse transcriptase for which, as for the assay, such activity is effectively stabilized by this approach, and nucleic-acid magnification since three kinds depend on existence of all enzyme activity (RNA dependent DNA polymerase, DNA dependent DNA polymerase, and RNaseH).
Although reverse transcriptase being freeze-dried under existence of the sucrose instead of trehalose under the same condition, and dealing in an additional experiment was shown, it was thought that trehalose was more slightly [as a freezing protection stabilizing agent / than a sucrose] excellent. (Please refer to an example 6.)
b. The reverse transcriptase under existence of a nonionic surfactant, and T7 Freeze drying of RNA polymerase In order to try to make precipitate of the protein under freeze-drying actuation into the minimum, maintaining enzyme activity during dialysis of an enzyme, coincidence dialysis was carried out and reverse transcriptase and RNA polymerase were made to freeze-dry under existence of a nonionic surfactant. six kinds of dialysis mixture -- the inside of the dialysis buffer solution -- 0%, 0.01%, 0.05%, 0.1%, 0.2%, and 0.5%TRITON It prepared so that X-102 might be included. The dialysis buffer solution is 20mM. HEPES, 0.1M NaCl, 0.1mM EDTA, 5mM NALC, 0
1mM zinc acetate and 0.2M trehalose were included. The last capacity of each dialysis mixture was 250ml. It is each buffer-solution 467 microliter MMLV-RT(2900 unit /mul) 46microl and T7 It mixed with 74micro [of RNA polymerase] (800 unit /mul) l, and initiation capacity of each dialysing fluid was set to 587microl. The sample was dialyzed at 4 degrees C to 60ml of corresponding buffer solutions, and the buffer solution of tales doses was exchanged 3 times. Although precipitate was seen by the 0%, 0.01%, and 0.05%TRITON X-102 content sample after the 3rd buffer-solution exchange, such precipitate is 0.1%, 0.2%, or 0.5%TRITON. It did not see by the X-102 content sample.
The capacity of each dialysing fluid was measured after dialysis, and the calculated enzyme concentration was adjusted suitably. Each sample is divided into four vials and it is MMLV-RT24,750 unit and T7 in each vial. RNA polymerase 11,000 unit was included. It freeze-dried as mentioned above. The appearance of the surfactant content freeze-drying object after desiccation is TRITON. It was undistinguishable from the freeze-drying object manufactured under un-existing [of X-102]. The vial was stored for 32 days at 4 degrees C and 55 degrees C after freeze drying.
The effectiveness over the enzyme activity of a nonionic surfactant was estimated by the magnification assay which uses a T7 gene 10 magnification system. Each freeze-drying enzyme preparation is made to rehydrate in the buffer solution for reconstruction, and it is MMLV-RT. 900 units and T7 RNA polymerase 400 unit was evaluated in each reaction mixture. The RNA gene 10 imprint object (100 copies / reaction) was used as a target-nucleus acid. Especially assay was performed as mentioned above, unless it refused. A result is reported by RLU.

表２

界面活性剤の存在下で３２日間貯蔵の凍結乾燥酵素の安定性						
	４℃で貯蔵			５５℃で貯蔵		
試料*	ＲＮＡ標的（二重反復試験）		標的なし	ＲＮＡ標的（二重反復試験）		標的なし
A	1612901	1317601	1543			
B	1151828	1146113	1700	791757	320417	1701
C	1286845	1219888	1544	1190527	905066	1690
D	1215264	1205790	1513	1251635	1388493	1513
E	1208586	1418260	1545	1245880	1052251	1591

* 試料Ａ＝－２０℃で貯蔵された非凍結乾燥酵素。

試料Ｂ＝０％TRITON X-102 中で凍結乾燥された酵素。

試料Ｃ＝０．１％TRITON X-102 中で凍結乾燥された酵素。

試料Ｄ＝０．２％TRITON X-102 中で凍結乾燥された酵素。

試料Ｅ＝０．５％TRITON X-102 中で凍結乾燥された酵素。

These results are TRITON. Nonionic surfactants, such as X-102, are MMLV-RT or T7. It is proved that formation of the protein precipitate after dialysis of RNA polymerase can be prevented effectively. A result is TRITON further. When X-102 does not do an operation harmful to magnification of a target-nucleus acid and moreover stores a freeze-drying enzyme at an elevated temperature, it is shown that it can act also so that enzyme activity with the passage of time may be stabilized further. A surface active agent does not cause the increment in background luminescence in this assay. These results are the samples (sample B) freeze-dried under un-existing [of a surfactant] again.

But it is proved that it has left the almost same activity as a non-freeze-drying enzyme. Further, the result shows not producing reduction of detectable activity by the freeze-drying enzyme preparation, when the long term storage of the freeze-drying enzyme preparation is carried out at an elevated temperature.

Probably, these results of suggest [other nonionic surfactants for example, say / getting that other surfactants of a BRIJ system, a TWEEN system and a TRITON system and the surfactant of a TERGITOL system are easily screened as mentioned above about those capacity to maintain desiccation protein in the state of fusibility during freeze drying, without having a bad influence on enzyme activity although not necessarily limited /, / directly] will be clear to this contractor.

Example 2: Coincidence freeze drying with reverse transcriptase, and RNA polymerase and a magnification reagent MORONI murine leukemia virus reverse transcriptase and T7 An RNA polymerase enzyme preparation Before drying, it is 50mM tris. - HCl (pH7.5), 0.1M NaCl, 0.1mM(s) EDTA, 1mM DTT, 0.01%(v/v) NP-40, or 0.1%(v/v) TRITON It held at -20 degrees C in the buffer solution for storage containing X-100 and 50% (v/v) glycerol.

For freeze drying, it is MMLV. RT 3x106 unit and T7 polymerase 1
Mix 3x106 unit (2.5ml of each preparation), and the permeable membrane of 12,000dalton of cuts off molecular weight is used. 20mM(s) HEPES (pH7.5), 5mM NALC, 0.1mM EDTA, 0.1mM zinc acetate, 0.2%(v/v) TRITON It dialyzed at 2-8 degrees C to the buffer solution of at least 50 capacity containing X-102 and 0.2M trehalose, and the buffer solution of this capacity was exchanged 3 times with the buffer-solution replace interval of at least 8 hours.
In 20ml of dialyzed enzyme preparations, they are 10.0mM spermidine, a 250mM imidazole / 150mM glutamic acid (pH6.8), and 99mM. It mixed with 60ml of magnification reagents containing dCTP of rATP of rCTP of NALC, 12.5%(w/v) PAP, and 12.5 mM(s) each and rUTP, and 31.2 mM(s) each and rGTP, and 10.0 mM(s) each, dGTP, dATP, and dTTP (capacity factor 6:2). Furthermore, it was shown that another experiment can be mixed without being accompanied by the result of being a capacity factor 7:1 (magnification reagent pair enzyme preparation), and having differed the reagent intentionally. theoretical -- a dialysis enzyme preparation and a magnification reagent -- an analogy -- being able to mix at a rate, the decision of the suitable ratio of a magnification reagent pair enzyme is fully within the limits of this contractor's capacity.
The enzyme with which it was mixed before freeze drying : the last presentation of a magnification reagent compound MMLVRT 2.7x106 unit and 1.2xT7 polymerase 106 unit, Each 6Xenzyme 106 unit, 5.0mM HEPES (pH 6.8-7.0), 0.025mM(s) EDTA, 0.025mM zinc acetate, 10.0mM spermidine, A 187.5mM imidazole, 112.5mM glutamic acid, 75.6mM NALC, 0.05%(v/v) TRITON X-102 or 9.4%(w/v) PAP (average molecular weight of 40,000dalton), It was dCTP, dGTP, dATP, and dTTP of 7.5 mM(s) each at rATP of rCTP of 0.05M trehalose and 9.4 mM(s) each and rUTP, and 23.4 mM(s) each and rGTP, and a list.
The mixed enzyme: Magnification reagent preparation (following enzyme: magnification reagent) 800 microliter was put in into each glass vial, respectively for freeze drying (all enzyme about 39,000 units /, vial). Freeze drying was performed like the case of an example 1. After freeze drying, the vial was processed, as shown by the following examples.
Example 3: Magnification activity assay of the freeze-dried reagent The newly [reverse transcriptase, RNA polymerase, and a magnification reagent] freeze-dried preparation was incubated by various time amount for three - 61 days in 25 degrees C, 35 degrees C, and 45 degrees C. Similarly all the vials were completely prepared from the same preparation. When directed, the vial containing a freeze-drying reagent was stored at -30 degrees C until drawing and the last samples were collected from the elevated temperature. The sample which shows the time of "zero" about each temperature was stored during [all] the experiment period at -30 degrees C.
When the vials at the time of the last are collected, it is a reagent for reconstruction (Reconstituting Reagent) (0.01%(v/v) TRITON X-102, 41.6mM MgCl2, 1mM ZnC2H3O2 or 10% (v/v) glycerol, 0) about all samples.
3% (v/v) ethanol, 0.02% (w/v) methylparaben, and 0.01% (w/v) propylparaben 1.5ml -- it rehydrated in inside and authorized about the capacity which causes nucleic-acid magnification of the contents of each vial.
The activity in a model magnification system was measured by the following approaches in this example. Each magnification reaction mixture included double-stranded-DNA restriction fragment 500 copy from the plasmid (PUC plasmid which has the 2.6kb fragmentation of a hepatitis B viral genome) which has a part of hepatitis B viral genome as a target-nucleus acid. Target DNA -- water -- or it diluted with 20micro of Homo sapiens blood serums l. Negative

control was similarly created except being target DNA non-**. This was added to 20micro [of 2X primer solution] l. The last presentation of this solution is 0.1Ns in the full capacity l of 40micro. KOH, 17.5mM EGTA, 25mM imidazole, 25mM glutamic acid, 0
They were 025% (w/v) Phenol Red and two kinds of primers of 0.3microeach M. from 3' target joint nucleotide array area complementary to DNA label (+) sense chain [the first primer ((-) sense)] -- changing -- and 5' -- an un-complementary part -- T7 It was located down-stream from the 5' un-complementary field which has the nucleotide sequence of the promotor of RNA polymerase. The second primer ((+) sense) had the nucleotide sequence which changes from a complementary target joint field to another DNA strand ((-) sense).
In 40micro of each reaction mixture l, it incubated at about 95 degrees C, and the double-stranded-DNA target was denatured. Next, the reaction was cooled for 5 minutes to the room temperature, and 10micro of buffer solutions l containing a 330mM imidazole and 200mM glutamic acid neutralized. If a target-nucleus acid is not DNA but RNA, this denaturation process will be unnecessary.
each reconfigurated enzyme:magnification reagent -- after adding 50 microliter to 50micro of DNA reaction mixtures l denaturalized and neutralized, it was incubated at 37 degrees C for 3 hours. Each reaction ended by 20microl (40 units) Adding RNase non-** DNase I.
The reconfigurated enzyme: Although it opted for relative magnification of each magnification reagent by using the homogeneity protection assay (HPA) indicated by Arnold and Nelson, and the U.S. Pat. No. 5,283,174 specification, it will be understood by this contractor that other assays using various detection means, such as a radioactive indicator, can be used. Contain acridinium ester indicator oligonucleotide BUROBU ((+) sense) of the about 75femto mol (fmol) designed so that it might be complementary to the RNA amplifier recon amplified by each magnification reaction. 10mM succinic-acid lithium (pH5.0), 2% (w/v) lauryl lithium sulfate, 1mM mercapto ethane sulfonic acid, 0.3%(w/v) PAP-40,230mM LiOH, 1.2M LiCl, 20mM EGTA, 20mM EDTA, a 100mM succinic acid (pH4.7), and 15mM(s) 100micro of 2 and 2' solutions l of - dipyridyl disulfide was added. It cooled, after mixing each test tube and incubating for 20 minutes at 60 degrees C. To each reaction mixture, they are 0.6M sodium borate (pH8.5) and 1%(v/v) TRITON. X-100 and 182mM(s) 300micro of solutions l containing NaOH was added, and the indicator which incubates for 6 minutes at 60 degrees C, and has not been combined with a hybridization probe was destroyed.
Cooling a reaction mixture for 5 minutes, the remaining chemiluminescence is 0.1%(v/v) H2O2 and a 0.1mM nitric acid. It is 1.0Ns immediately after carrying out automatic impregnation of the 200microl. It measured after pouring in NaOH in RUMINO meter (LEADER (trademark) Gen Probe, Incorporated (Gen-Probe Incorporated), Sun Diego, CA). The amount of the light emitted succeedingly is reported per relative light (RLU). The background level of luminescence was within the limits of about 2000 to 4000 RLU under these conditions.
As the result was recorded and it was shown below, it displayed about each storage temperature (25 degrees C, 35 degrees C, and 45 degrees C). Each sample was authorized and averaged by the Mie iteration trial. The data about each temperature were plotted in the graph using this average. Drawing 1 corresponds to Table 3 and, in drawing 2, Table 4 and drawing 3 correspond to Table 5.

表3

凍結乾燥酵素：増幅試薬の安定性　貯蔵温度２５℃							
貯蔵日数	0	11	16	20	30	40	61
ＤＮＡ標的不含試薬（ＲＬＵ）	2053 2130 2148	1911 1590 1752	1524 1561 2037	2188 1990 1606	1851 1847 1923	1548 1726 2382	1972 1655 1538
平均ＲＬＵ	2110	1751	1707	1928	1874	1885	1722
ＤＮＡ標的含有試薬（ＲＬＵ）	1562029 1756224 1070164	2105440 1903081 1492458	1248988 1509929 1944566	2129935 2363198 1922529	1927067 1422699 1274124	1417883 1601071 1889588	1486111 1290950 1210344
平均ＲＬＵ	1462806	1833659	1567828	2138554	1541297	1636181	1329135
ヒト血清中試薬， ＤＮＡ標的不含（ＲＬＵ）	8437 3902 3534	2904 2893 3003	2660 2993 2768	3044 3152 2951	2919 2971 2379	2465 3089 2958	2946 3473 3686
平均ＲＬＵ	5291	2933	2807	3049	2756	2837	3368
ヒト血清中試薬， ＤＮＡ標的含有（ＲＬＵ）	1955525 2255411 2282281	2282336 2204415 2206778	2282171 1860043 1903519	1760428 1992765 2093235	2034705 2101999 2064041	1936366 1770109 1811820	1643624 1762360 1622750
平均ＲＬＵ	2164406	2231176	2015244	1948809	2066915	1839432	1676245

表4

凍結乾燥酵素／増幅試薬の安定性
貯蔵温度３５℃

貯蔵日数	0	3	9	16	21	50	61
ＤＮＡ標的不含試薬（ＲＬＵ）	2429	17989	1768	1878	2378	1430	1559
	2203	1775	1649	1919	2330	1411	1566
	1996	1891	1840	2043	1995	1338	1692
平均ＲＬＵ	2209	7218	1752	1947	2234	1393	1606
ＤＮＡ標的含有試薬（ＲＬＵ）	1173260	2310573	2186899	1559681	1876363	1458120	1366068
	1580018	2136598	2119044	1385165	1919833	1932847	1443874
	1389614	2303010	1568334	1632416	1979406	1343433	1421081
平均ＲＬＵ	1380964	2251060	1958092	1525754	1925201	1578133	1410341
ヒト血清中試薬, ＤＮＡ標的不含（ＲＬＵ）	4819	3298	3608	3575	2912	3074	3836
	4779	9577	3200	3535	3422	3044	4160
	24541	3349	3114	3712	3151	3027	3901
平均ＲＬＵ	11380	5408	3307	3607	3162	3048	3966
ヒト血清中試薬, ＤＮＡ標的含有（ＲＬＵ）	1946881	2228745	2233566	2087936	1984355	2255784	1873070
	2158003	2289829	2303812	2163922	2192597	2147927	1789954
	2110796	2286956	2179206	2152655	2121658	2087549	2049762
平均ＲＬＵ	2071893	2268510	2238861	2134838	2099537	2163753	1904262

表5

凍結乾燥酵素／増幅試薬の安定性 貯蔵温度４５℃					
貯蔵日数	0	6	11	16	33
ＤＮＡ標的不含試薬（ＲＬＵ）	2508 2250 2159	1613 1872 1903	1687 1781 2206	2626 2027 2056	1594 1596 1661
平均ＲＬＵ	2306	1796	1891	2236	1617
ＤＮＡ標的含有試薬（ＲＬＵ）	1431296 1329706 1288191	1097084 949892 798877	975001 758705 1242188	1320113 939417 972442	1017853 1368153 1015174
平均ＲＬＵ	1349731	948618	991965	1077324	1133727
ヒト血清中試薬， ＤＮＡ標的不含（ＲＬＵ）	3554 3109 4239	3375 4452 2960	3011 3119 3382	3068 3559 3381	3183 3115 2826
平均ＲＬＵ	3634	3596	3171	3336	3041
ヒト血清中試薬， ＤＮＡ標的含有（ＲＬＵ）	1663770 1677985 1747637	1850263 1868747 2016609	1691590 1684565 1646303	1691372 1709387 1765393	1615426 1913706 1799445
平均ＲＬＵ	1696464	1911873	1674153	1722051	1776192

The coincidence freeze-drying enzyme with which these data were manufactured by the

approach of a publication in this specification: Holding the enzyme activity [all (RNA dependent DNA polymerase, DNA dependent DNA polymerase, RNaseH, and RNA polymerase) four kinds] which a magnification reagent needs for performing nucleic-acid magnification according to the used imprint medium nature amplifying method is shown. Therefore, the data shows that the remarkable adverse reaction to other components of the arbitration of nucleotide triphosphoric acid or a magnification reagent does not exist, when carrying out coincidence freeze drying of the reagent together with reverse transcriptase and RNA polymerase.

When, as for these results, a magnification reaction is further performed under existence of a complicated biological material, such as a Homo sapiens blood serum, the enzyme activity and RNA polymerase enzyme activity of reverse transcriptase show not being prevented intentionally. Therefore, it is thought that the freeze-drying magnification reagent is suitable for using together with a sample which is obtained by clinical-diagnosis setup.

The data can be interpreted by many approaches, and using the format of the Arrhenius' equation, one of the more useful interpretation means predicts the stability of the constituent over a long period of time rather than it is actually examined. Generally the Arrhenius' equation is used for predicting the stability of the rate of a chemical reaction, and various thermolability compounds as a function of temperature by this contractor.

The Arrhenius' equation is active oxygen (or reagent) so that it may be used in this specification. The first order reaction of enzyme (or reagent) deactivation which has a single deactivation mechanism with a single deactivation rate and a total test temperature at ********** temperature is assumed. Formula -- the applicant used -- ln(k2/k1) = (-Ea/R (T2-T1)) (/(T2xT1)) It comes out, and it is, k2 is a rate constant in experiment temperature (degree K) among a formula, k1 is the rate constant of the reaction in contrast temperature, Ea is the activation energy of a reaction, R is a gas constant (1.987cal / **K-mol), and T1 is contrast temperature (for example, 298.16 degrees K (25 degrees C)), and T2 is experiment temperature (expressed with degree K).

If it is assumed that Ea(s) are 15,000cal / mol and contrast and experiment temperature are known, the ratios k2/k1 of a rate constant can be determined. When [easy] both contrast and experiment temperature are 25 degrees C, since these constant ratios have the the same constant, they are 1. A prediction ratio will be 2.27, when experiment temperature is 35 degrees C and contrast temperature is 25 degrees C. A prediction ratio will be 4.91, when experiment temperature is 45 degrees C and contrast temperature is 25 degrees C. The ratio is 30.33, when contrast temperature is 5 degrees C using the same formula and experiment temperature is 45 degrees C.

the paddle gap whose storage time amount of it the rate constant ratio is expressed with the time, or is a day, a week, etc. -- an imitation -- the "decomposition ratio" of experiment storage time amount pair standard storage time amount can be considered. Therefore, when a freeze-drying enzyme / magnification reagent will decompose to 90% of the capacity of the origin of it in 45 degrees C in 30 days, the Arrhenius' equation expects that 147.3 (30x4.91) days will be taken for activity to fall similarly at 25 degrees C.

Therefore, as for the data, it is proved that the component with which the freeze-drying preparation was mixed does not lose notably those capacity that supports magnification also after 30 days by the "real time" at 45 degrees C. Furthermore, even if these data actually carry out storage for 2.5 years (30.33x 30 days) at five months or 5 degrees C almost by 25 degrees C by using the Arrhenius' equation before using a freeze-drying reagent, the reagent expects that

activity will not lose intentionally.
Although an applicant shows these date analysis methods as what helps an understanding of this invention, he does not desire to be restricted or restrained by theoretical consideration. The actual stability of the constituent of this invention may differ from prediction of the Arrhenius' equation which gives the general index about prediction of the stability of a freeze-drying reagent.
Example 4: T7 of a freeze-drying reagent RNA polymerase assay The freeze-drying enzyme manufactured in the example 2: The magnification reagent was incubated at 35 degrees C for zero day, three days, nine days, 16 days, 21 days, and 30 days. It stored at -30 degrees C until drawing and the last samples were collected from the stress temperature in the vial at each [these] time.
RNA polymerase activity was measured by reconfigurating each aliquot of a freeze-drying reagent in 1.5ml (0.01%(v/v) TRITON X-100, 41.6mM MgCl2, 1mM ZnC2H3O2 or 10% (v/v) glycerol, 0.3% (v/v) ethanol, 0.02% (w/v) methylparaben, and 0.01% (w/v) propylparaben) of buffer solutions for reconstruction. Next, it is 20mM about the reagent. HEPES (pH7.5), 5mM NALC, 0.1mM EDTA, 0.1mM ZnC2H3O2, 0.1M NaCl and 0.2% (v/v)
TRITON It diluted with the solution containing X-102 100 times, 200 times, and 400 times. 22mM(s) The reaction premix containing CPT and UTP of ATP and GTP of MgCl2 and 7.8 mM(s) each, and 2.5 mM(s) each, 62.5mM tris (pH7.5), 2.5mM spermidine, and a 0.5-nanomole target-nucleus acid was prepared separately. A target is the shape of a straight chain pUC which has T7 promotor immediately located in the upstream from bacteriophage T7 gene 10. It was T7G10 plasmid. This plasmid originated in plasmid pGEMEX-1 (a pro megger corporation, Madison, WI).
The reaction premix was divided into 40microl aliquot, and each aliquot was incubated for 3 minutes at 37 degrees C. Enzyme: In addition to the premix test tube which warmed each diluent 10 microliter of a magnification reagent, it incubated for 20 minutes at 37 degrees C. Contain acridinium ester marker gene 10 oligonucleotide probe ((-) sense) of the about 75femto mol designed so that it might be complementary to a transcript. 10mM succinic-acid lithium, 2% (w/v) lauryl lithium sulfate, 1mM mercapto ethane sulfonic acid, 0.3%(w/v) PAP-40,230mM LiOH, 1.2M LiCl, 20mM EGTA, 20mM EDTA, a 100mM succinic acid (pH4.7), and 15mM(s) 50micro of 2 and 2' solutions l of - dipyridyl disulfide was added to each test tube. The HPA process was made to include the standard sample which contains the single stranded DNA of a complementary 10femto mol (fmol) in gene 10 probe, and the quantum of the amount of RNA produced in the experiment reaction mixture was carried out. Hybridization was essentially performed like [in the case of an example 2] except hybridization capacity having been one half. The remaining acridinium ester was made to react after disassembling the indicator by which hybridization was not carried out, and luminescence was measured as RLU in RUMINO meter. The raw data was changed into the unit of per mul of RNA polymerase activity as follows. Hara RLU obtained about the forward contrast reaction was deducted from RLU obtained by negative contrast (target DNA non-**). This numeric value is the amount of net luminescence obtained when RNA of 10 fmol is in a sample, and can be expressed as RLU/fmol RNA. Similarly, RLU obtained about each sample can be deducted from background luminescence (for RLU / 20 minutes). When this numeric value is broken by the numeric value (RLU/fmol RNA) which was able to be acquired about the criterion, that result is the fmol number of RNA produced in per for 20 minutes in each reaction. Since RNA polymerase activity 1 unit was defined as generation of 1 fmol RNA for [of a under / assay conditions] 20 minutes, this numeric value is also the number of unit of the RNA polymerase activity for every 10microl capacity of the enzyme added

first.
The 1.5ml original reconstruction enzyme in which the data obtained from these reactions were first shown with each test tube in fmol of generated RNA: It was plotted about each time amount of storage by expressing as a function of the number of microliter of a magnification reagent at 35 degrees C. The easy linear function was drawn. When data were plotted, the line which suited to each data obtained about the time best was calculated, and the inclination of this curve was expressed as the unit/microliter of T7 polymerase activity. When it considers that the "time of zero" time is 100% of activity, it is T7 at the time of each remainder. The unit by which RNA polymerase was calculated was expressed as remaining activity %.
Drawing 4 is T7 in a freeze-drying enzyme:magnification reagent as a function of the days of 35-degree C storage. It is the plot of the number of unit/microliter of RNA polymerase. This result is T7. Even if there is reduction in RNA polymerase, hardly happening over the incubation period for 30 days at 35 degrees C is shown.
Example 5: Reverse transcriptase assay of a freeze-drying reagent The activity of the freeze-drying MMLV reverse transcriptase with which it incubated at 35 degrees C for three days, nine days, 16 days, 21 days, and 30 days was authorized as follows. It stored at -30 degrees C until drawing and the last sample were brought together in the time amount of assignment of each vial from the stress temperature.
Like the case of an example 4, each vial was reconfigurated in 1.5ml of buffer solutions for reconstruction, and was diluted 100 times, 200 times, and 400 times. About separate reverse transcriptase premix mixture, they are dATP of 5mM KCl and 0.25 mM(s) each, dTTP, dCTP and dGTP, and 62.5mM tris (pH7). MgCl2, 30mM
It manufactured so that 5), 2.5mM spermidine, 3.75nM targets RNA, and the magnification primer of 750nM(s) might be included. Target RNA was the T7 gene 10 RNA transcript produced in the example 4. The primer was 22 base die-length oligonucleotide designed so that hybridization might be carried out to the field near Target's DNA three-dash terminal. Enzyme diluent 10 microliter was added to reaction premix 40microl of Hikami, respectively. The reaction was performed at 37 degrees C by the incubation for 15 minutes. Each reaction was stopped by addition of acridinium ester indicator hybridization probe 50microl. The probe was designed so that it might be complementary to newly compounded gene 10cDNA.
Detection by HPA was performed like a publication in the example 3. The result was measured by RLU.
This assay measured the RNA-dependent-DNA-polymerase activity and RNase H activity of MMLV reverse transcriptase. Since a probe cannot carry out hybridization to cDNA without decomposition of the RNA chain of the RNA:DNA hybrid produced by expanding of gene 10 primer, the latter activity is measured indirectly.
One unit of the these-mixed enzyme activity was defined as detection of 1 fmol cDNA for [of a under / the above-mentioned reaction condition] 15 minutes. Count of the unit of the remaining enzyme activity in each time and dilution was performed like the case of an example 4, using the magnification cDNA of 10 fmol as a criterion.
Drawing 5 is the plot of the number of unit/microliter of RT activity in a freeze-drying enzyme:magnification reagent as a function of 35-degree C storage days. This result shows hardly happening over the incubation period for 30 days at 35 degrees C, even if there is a fall of RT activity.
Example 6: Coincidence freeze drying with reverse transcriptase and RNA polymerase, a nucleotide, and a primer The front example illustrated the manufacture and use of a single

reagent which contain RNA polymerase and the dry-maintenance-of-idle-boiler preparation of reverse transcriptase, and nucleotide triphosphoric acid and a subfactor required for nucleic-acid magnification together. If the capacity of such a "single vial" reagent that amplifies a nucleic acid is after hot and long-term storage The number of containers of the kit for nucleic-acid magnification so that a routing counter may be decreased in the approach of using such a reagent Three pieces So that two pieces (for example, a freeze-drying enzyme / primer / magnification reagent, and the reagent for reconstruction) may be decreased from for example, (a freeze-drying enzyme:magnification reagent, a primer, and the reagent for reconstruction) Probably, it will be clear to this contractor that it is possible **** easily to make one kind or two or more magnification primers include in a freeze-drying preparation.
such a preparation -- the first target-nucleus acid -- RNA -- it is -- and the amplifying method -- the [the thing of isothermal, for example, KASHIAN, FURUTSU, and / PCT public presentation] -- the [WO 91/No. 01384 or KASHIAN et al., and / PCT public presentation] -- like [in the case of being the approach / like / in WO 93/No. 22461], when a magnification reaction does not use the temperature which denaturalizes the enzyme of one side or both, it is useful.
Example 7: Freeze drying with reverse transcriptase and a sucrose The applicant discovered further that a sucrose (for example, 0.2 M concentration) could be used as a freezing protection stabilizing agent in freeze drying of reverse transcriptase. The preparation which was considered that the stabilization effect of a sucrose is good, and contained MMLV-RT, and was freeze-dried in the 0.2M sucrose at -20 degrees C in 50% (v/v) glycerol as compared with the same standard liquid solution by which time amount storage was carried out maintained 93% of the activity of a standard MMLV-RT preparation for the freeze-drying object after storage for 30 days at 4 degrees C. The freeze-drying object containing not a sucrose but 0.2M trehalose processed similarly showed an average of 105% of activity about the criterion under the same conditions.
example 8 :P Freeze drying under existence of AP the case where, as for an applicant, a polyvinyl pyrrolidone (PAP) is further mixed with trehalose in the buffer solution before freeze drying -- an enzyme:magnification reagent -- trehalose -- the case where it is made to freeze-dry under an independent existence -- large -- freeze drying T7 RNA polymerase: -- it discovered raising the stability of a MMLV-RT:magnification reagent preparation. this surprising knowledge -- the stability of a freeze-drying enzyme:magnification reagent -- as a freezing protection stabilizing agent -- trehalose -- independent -- or it rubs from the case of the freeze-drying constituent containing the combination of trehalose and PAP -- carry out -- by using PAP independently, it is almost the same or suggests it being maintained by high extent and getting. Freeze drying of an enzyme is TRITON as explained in full detail in the example 2. It can optimize by dialyzing a purification enzyme to the dialysis solution containing X-100 or another nonionic solubilizing agent. This dialysis solution does not contain trehalose. The aliquot of an enzyme solution can be prepared after a buffer-solution exchange process, and PAP of various amounts can be added to each aliquot. Next, to those aliquots, as explained in full detail in the example 2, an enzyme:magnification reagent can be added and it can freeze-dry. These freeze-drying samples can be authorized at different temperature like the case of an example 3 about various capacity of the reconfigurated reagent which supports nucleic-acid magnification about each enzyme activity by carrying out time amount incubation.
It will be understood by this contractor that the upper example is not for having only indicated the approach of this invention and the desirable embodiment of a constituent, and restricting or defining invention. Other embodiments are included by the claim following these examples.

配列表

（1）一般情報：

（i）出願人：ジェン・プローブ・インコーポレーテッド (Gen-Porbe Incorporated)

（ii）発明の名称：核酸増幅用の安定化された酵素組成物

（iii）配列の数：3

（iv）宛先：

(A) 住所：ライアン・アンド・ライアン (Lyon &Lyon)

(B) 地区：６３３ウェスト・フィフス・ストリート・スィート (West Fifth Street Suite) ４７００

(C) 市名：ロサンゼルス

(D) 州名：カリフォルニア

(E) 国名：米国

(F) 郵便番号：９０７１－２０６６

（v）コンピューター読取り形式：

(A) 中型：ディスケット

(B) コンピューター：ＩＢM適合性

(C) 操作システム：ＤＯＳ

(D) ソフトウェア：ファストセク・バージョン (FastSEQ Version) １．５

（vi）現行出願資料：

(A) 出願番号：不明

(B) 出願日：

(C) 分類：

（vii）先行出願資料：

(A) 出願番号：

(B) 出願日：

（viii）弁理士／代理人情報：

(A) 氏名：シェルドン　Ｏ．ヒーバー (Sheldon O. Heber)

(B) 登録番号：３８，１７９

(C) 照会／事件整理番号：２１１／１２７－ＰＣＴ

(ｉｘ) 通信情報：

(A) 電話：２１３－４８９－１６００

(B) ファクシミリ：２１３－９５５－０４４０

(C) テレックス：

(2) 配列番号：１の情報：

(ｉ) 配列の特徴：

(A) 長さ：４８塩基対

(B) 型：核酸

(C) 鎖の数：１本

(D) トポロジー：直鎖状

(ｉｉ) 配列の種類：ｃＤＮＡ

(ｉｉｉ) ハイポセティカル：なし

(ｉｖ) アンチセンス：なし

(v) フラグメント型：

(ｖｉ) 起源：

(ｘｉ) 配列：配列番号：１：

AATTTAATAC GACTCACTAT AGGGAGAGAG AAGTGGTCAC GGAGGTAC 48

(2) 配列番号：２の情報：

(ｉ) 配列の特徴：

(A) 長さ：２２塩基対

(B) 型：核酸

(C) 鎖の数：１本

(D) トポロジー：直鎖状

(ｉｉ) 配列の種類：ｃＤＮＡ

(ｉｉｉ) ハイポセティカル：なし

(ｉｖ) アンチセンス：なし

(v) フラグメント型：

(ｖｉ) 起源：

（ｘｉ）配列：配列番号：２：

CATGACTGGT GGACAGCAAA TG 22

（２）配列番号：３の情報：

（ｉ）配列の特徴：

（Ａ）長さ：２６塩基対

（Ｂ）型：核酸

（Ｃ）鎖の数：１本

（Ｄ）トポロジー：直鎖状

（ｉｉ）配列の種類：ｃＤＮＡ

（ｉｉｉ）ハイポセティカル：なし

（ｉｖ）アンチセンス：なし

（ｖ）フラグメント型：

（ｖｉ）起源：

（ｘｉ）配列：配列番号：３：

CTGCTGGAGA TAAACTGGCG TTGTTC 26

[Translation done.]